Isocitrate Assay Kit (Colorimetric)

Isocitrate Assay Kit (Colorimetric) is a detection kit for the quantification of Isocitrate in cell lysate, tissue homogenate, serum, food and beverage.

Catalog number: ARG82172

Package: 100 tests

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INTRODUCTION

Isocitric acid is a structural isomer of citric acid. Salts and esters of isocitric acid are known as isocitrates. The isocitrate anion is a substrate of the citric acid cycle. Isocitrate is formed from citrate with the help of the enzyme aconitase, and is acted upon by isocitrate dehydrogenase.

Isocitric acid is commonly used as a marker to detect the authenticity and quality of fruit products, most often citrus juices. In authentic orange juice, for example, the ratio of citric acid to D-isocitric acid is usually less than 130. An isocitric acid value higher than this may be indicative of fruit juice adulteration. [Provide by Wikipedia: Isocitric acid]

PRINCIPLE OF THE ASSAY

This Isocitrate Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of isocitrate present in food, beverage and biological samples. This assay measures the NADPH generated from the oxidation of isocitrate. The NADPH converts the dye to an intense violet color with an absorption maximum at O.D. 565 nm. The increase in absorbance at O.D.565 nm is directly proportional to the isocitrate concentration.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
Enzyme A	120 µL	-20°C
Enzyme B	120 µL	-20°C
NADP/MTT	1 mL	-20°C
Standard	1 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 565 nm
- Clear flat-bottom 96 well microplate
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum for assay.

Tissue: Rinse tissue in 1X PBS (pH 7.4) to remove blood. Homogenize tissue (50 mg) in about 200 µL of buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 14,000 x g for 15 minutes at 4°C. Collect the supernatant for assay.

Cell lysate: Collect cells by centrifugation at 2,000 x g for 5 minutes at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 14,000 x g for 10 minutes at 4°C. Collect the supernatant for assay.

Note: All samples can be stored at -20 to -80°C for at least one month.

REAGENT PREPARATION

- **Working Reagent:** for each assay, mixing 8 µL of NADP/MTT, 1 µL of Enzyme A, 1 µL of Enzyme B and 75 µL of Assay Buffer. Prepare immediately before assay.
- **Standards:** Prepare 200 µL of 5000 µM Premix by mixing 10 µL of the Standard (100 mM) and 190 µL of distilled water. Dilute standards as follows.

Standard tube	Isocitrate (µM)	Distilled water (µL)	Standard Premix, 5000 µM (µL)
S1	5000	0	100
S2	3000	40	60
S3	1500	70	30
S4	0	100	0

ASSAY PROCEDURE

Keep thawed Enzyme A and B on ice and equilibrate all other reagents to room temperature (25°C). Briefly centrifuge tubes before use.

	Standard well	Sample well
Standard	20 µL	
Sample		20 µL
Working Reagent	80 µL	80 µL
Tap plate to mix briefly and thoroughly. Incubate for **10 minutes** at **room temperature**.		
Read the absorbance at **O.D. 565 nm**.		

CALCULATION OF RESULTS

1. Subtract blank value (distilled water, S4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the isocitrate concentration of Sample as follows:

   Isocitrate (µM) = [(OD_{Sample} – OD_{Blank}) / Slope (μM^{-1})] x n

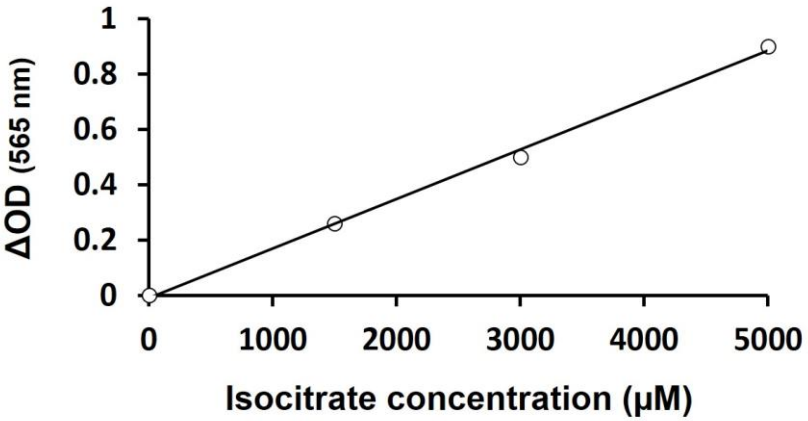
   Note:

   - OD_{Sample}, OD_{Blank}: the O.D. 565 nm values of the sample and distilled water blank (S4), respectively.
   - n: the sample dilution factor.

2. Unit conversion: 1 µM is equiv. to 189 µg/L or 0.189 ppm isocitrate.
3. If the calculated concentration is higher than 5000 µM, dilute sample in water and repeat assay. Multiple the result by the dilution factor.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Isocitrate Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

20 µM